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A Possible Pathophysiological Explanation

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ENHANCED ATP RELEASE FROM THE UROTHELIUM OF PATIENTS WITH PAINFUL BLADDER SYNDROME: A POSSIBLE PATHOPHYSIOLOGICAL EXPLANATION.

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ABSTRACT

Purpose:

The aim of this study was to establish the level of ATP release by the urothelium from patients with painful bladder syndrome and compare it with that from the normal human urinary bladder.

Methods:

Biopsies of urothelium, obtained from patients with painful bladder syndrome were subjected to stretch (by 130 and 150% of the original length) and electric stimulation (10Hz). A luciferase assay was used to quantify ATP release. The neurotoxin, tetrodotoxin was used to block the neuronal source of ATP release.

Results:

There was a significantly greater release of ATP following mechanical stretch of the urothelium from painful bladders compared with control bladders. The increase in ATP release was statistically significant whether expressed in absolute values (3791.4±667.9 pM/g of tissue compared with 77.6±16.2 pM/g of tissue for controls) or as the increase over basal levels (182.2±24.8 % increase above baseline compared with 75.4±21.7 % over basal levels for controls).

Similarly, there was a significant release of ATP following electric field stimulation of the urothelium from painful bladders (1348.6±278.2pM/g of tissue) compared with normal (61.7±10.1 pM/g of tissue) [$p<0.005$], a 730.4±45.8 % increase above the basal level compared with an increase of 37.9±4.4 % for normal bladders [$p<0.001$]). The source of ATP release was mainly non-neuronal, 91 % for the painful bladders and 81 % for controls.

Conclusions:

There is a significantly increased level of ATP release from the urothelium of painful bladders in comparison to normal bladders suggesting an important potential functional role for ATP in this condition.

INTRODUCTION

Adenosine triphosphate (ATP) has been increasingly recognised as an important sensory neurotransmitter. A number of studies have supported the idea that mechanical stimuli can evoke the release of ATP from epithelial cells lining "tubes" or "sacs" such as the urinary bladder [1]. This extra-cellular ATP, which is most likely urothelial in origin, has been implicated in the distension-evoked activation of bladder afferents [2]. Once released from epithelial cells after bladder stretch, ATP is thought to activate purinergic receptors on suburothelial sensory nerves and thus may play an important role in sensory functions such as nociception [3].

Further support for this theory comes from the study of mice lacking the $P2X_3$ sensory purinergic receptor subunit (normally expressed by a subset of bladder afferents), which exhibit normal distension-evoked urothelial ATP release but diminished reflex bladder contractions and voiding behavior and a reduction in the behavioural (pain) response to the injection of ATP [4]. Based on these observations it has been suggested that mechanical stretch evoked ATP release from the urothelium may play a major role in both volume and pain-mediated reflexes. Suggesting that the purinergic system is important not only in normal bladder function but may have an important pathogenic role in functional disorders of the bladder.

Patients with painful bladders form a heterogeneous group based on a diagnosis of exclusion rather than a precise patho-physiology. This study was aimed to establish the level of ATP release by the urothelium from painful bladders and compare it with that from the normal human urinary bladder.

METHODS

The experiments were performed using tissue from the bladder dome. Patients were diagnosed as having painful bladder syndrome on the basis of the symptoms of frequency due to bladder pain, leading to a reduced functional capacity but in the absence of urodynamically assessed detrusor overactivity. The urothelium from painful bladders

was obtained by cold cup biopsy in patients undergoing cystodistension following full informed consent and approval from the local ethics committee (n=8). The urodynamic studies in this group showed a stable bladder during the filling phase but with small capacity and perception of pain with further filling. These patients had a mean maximum cystometric capacity of 206mL (range=168-280mL). Cystoscopy in these patients showed no obvious bladder pathology. Control bladders were obtained from patients with urodynamically proven stable bladders undergoing surgery for stress urinary incontinence (n=4) and from patients undergoing cystectomy for cancer who had no history of receiving radiotherapy or any form of intravesical treatment and had no lower urinary tract symptoms that would suggest a functional bladder disorder (n=5). The tissue was taken from a site distant to the tumour from a normal looking bladder area.

Tissues were set up under 1g tension in 2.5mL tissues baths containing Krebs bicarbonate solution (composition in mM/L: NaCl 118.4, $NaHCO_3$ 24.9, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4$ 1.15, KH_2PO_4 1.15, glucose 11.7) at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2 The tissues were placed between two platinum-wire loop-electrodes and, using suture silk, the bottom end of the tissues were secured to a tissue holder. The upper ends were attached (with suture silk) to isometric force transducers (UFI model 1030). The transducers were connected to a Cambridge Electronic Design (CED) data acquisition system, allowing changes in isometric tension to be recorded. Tissues were equilibrated for at least one hour with the bathing medium changed at 15-minute intervals. This washing removed the artefact of increased ATP release caused by surgical trauma. The urothelium was then mechanically stretched by 130% and 150% of the original length and bath samples taken 2 minute following the stretch for quantification of ATP release. The tissues were also subjected to electric field stimulation (EFS) at 10Hz (40V, 0.2msec pulse-width for 60s) using a Digitimer Powerstim Stimulator and ATP release assessed [5].

To enable the calculation and separation of neuronal and non-neuronal sources of ATP, release induced by mechanical and electrical field stimulation was determined following a 20 minutes incubation of the tissues with the neurotoxin, tetrodotoxin (5µM). All the

tissues were weighed at the end of each experiment. The ATP released was expressed in pM per gram of tissue and as percentage increase above the basal (resting) level.

Luciferase Assay: ATP release was quantified by luminometry by the luciferin-luciferase assay using ATP reagent HS (BioThema, Haninge). 50µl of the ATP standards, the samples and the blank (for measuring the background luminosity) were placed in a 96-well microplate (NUNC^{TM}, Denmark), which was followed by automatic addition of 100µl of luciferin-luciferase reagent in the 'Lucy Anthos 1' luminometer (Anthos Labtech Instruments, Austria). The analysis was performed using 'Stingray' software (Dazdaq Ltd, UK). The ATP concentration was calculated from calibration curves constructed from the ATP standards with correlation coefficient varying from 0.998 to 0.999. The increase in ATP was expressed as the percentage rise above the baseline or as amount of ATP in pM per gram of tissue.

Statistical analysis was performed using the Student's t-test, with $P<0.05$ being taken as significant.

RESULTS

At the start of the experiment the basal level of ATP in the bath before stretching or electrically stimulating the tissue was 45.7±4.9pM g^{-1} tissue. Mechanical stretch of the urothelium increased ATP levels by 108.4±19.3 % following 130% stretch and by 182.2±24.8 % following a 150% increase in tissue length (Figure 1, Table 1). Electrical field stimulation also resulted in an enhanced ATP release from the urothelium, 10Hz stimulation increasing ATP bath concentrations by 730.4±45.8 % (Table 2). The presence of tetrodotoxin (5µM) did not significantly affect ATP release induced by stretch indicating a non-neuronal source of ATP (p=NS). In contrast, electrical field stimulation induced release was reduced by 79% in the presence of this neurotoxin ($P<0.005$) (Table 2).

For urothelium from painful bladders the basal release of ATP was greatly enhanced, bath concentrations rising from 45.7±4.9 pM g^{-1} to 1321.1±475.7 pM g^{-1} tissue ($P<0.01$, n=8). Following mechanical stretch the difference from control tissue was even greater and the release of ATP induced by a 130% stretch was 2944.6±565.9 pM g^{-1} compared with only 67.5±13.2 pM g^{-1} for control tissue ($P<0.001$). Similarly for the 150% stretch, ATP release was greatly enhanced over that of control tissue, mechanical stimulation increasing ATP release to 3791.4±667.9 pM g^{-1} in the painful bladders as compared to 77.6±16.2 pM g^{-1} for control tissues ($P<0.001$). This large increase in ATP release was statistically significant whether the data were expressed in absolute values, as the increase above basal levels or as a percentage of the initial basal release (Figure 2, Table 2). As with the control tissues, ATP release was not significantly altered in the presence of tetrotodoxin (5μM) indicating a non-neuronal origin.

Following electrical field stimulation the release of ATP from the urothelium of painful bladders was large (730.4 ± 45.8% above basal levels) and this was significantly greater than that obtained in control tissues. This enhanced release was statistically significant whether expressed in absolute values, the increase above basal levels or as a percentage increase over basal levels (Figure 2, Table 2). As for control tissues, the ATP release induced by electrical field stimulation was significantly reduced ($P<0.05$) in the presence of tetrodotoxin; this inhibition (75%) being similar to that obtained for control tissue (78%).

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DISCUSSION

There has been an increasing awareness that normal bladder contraction may be more significantly controlled by afferent neuronal activity than previously recognised. Whilst in a normal bladder, the afferent activity is mediated via the Aδ-fibres [6], in pathological conditions like painful bladder syndrome, it may occur via normally inactive C-fibres [7]. In theory, increased afferent activity could be of great importance in the development of

painful bladder disorders, where the bladder is hypersensitive to stretch and the resulting sensation may be perceived as pain.

Several studies have supported the important afferent sensory role for ATP in the bladder. In rats, it has been reported that there is a significant reduction in the pelvic afferent nerve activity (by 75%) in bladders exposed to intravesical α-β-methylene ATP that acts by desensitising the purinergic (P_2X) receptors [8]. If ATP is a sensory neurotransmitter, then conceptually, an increased purinergic activity may lead to a condition where the bladder is oversensitive to distension resulting in so called 'painful bladder syndrome'.

Painful bladder syndrome/Interstitial Cystitis (IC)" is a broad term that implies painful distension of the bladder and includes a wide spectrum of pathological conditions [9]. There is a very sparse data available regarding the pharmacology of this disorder mainly because of limited knowledge about its patho-physiology and most theories about the patho-physiology of this condition have not been rigorously tested.

The atropine resistant contractile component in detrusor strips from patients with so called "interstitial cystitis" has been shown to be about 43% of the total response, while this component has not been observed in controls [10]. Recent studies have suggested an increased release of ATP from the urothelium of cats with 'interstitial cystitis' (feline interstitial cystitis; FIC), which are used as an animal model for 'interstitial cystitis' [11] and also from the cultured human urothelium and urine from patients with 'interstitial cystitis' [12, 13]. In this study, we used the urothelium from patients with painful bladders, which was stimulated using mechanical stretch and electric field stimulation and the consequential ATP release was quantified. There was a significantly increased release of ATP from this urothelium following both electric field stimulation as well as mechanical stretch, in comparison to the normal bladders. If ATP is a sensory neurotransmitter in bladder responsible for bladder sensation and nociception, as suggested by Burnstock et al (2000) [3] and Ferguson et al (1999) [14], this remarkably increased release of ATP from the urothelium of painful bladders would explain the different symptoms resulting from this disorder namely: bladder hypersensitivity, pain, smaller functional bladder capacity and the resulting urinary frequency along with urgency. However, the increased ATP release may not be the only purinergic component

responsible for this condition; the distribution and quantity of the sensory purinergic receptors ($P2X_3$) may also contribute.

Furthermore, the major source for ATP release following mechanical stretch, which is probably a more physiological stimulus, is non-neuronal. This further supports the view that it is the urothelially released ATP, which is principally responsible for bladder sensation as suggested by Ferguson (1999) [14] or an increased bladder sensation as in this case.

This study provides a direct evidence of significantly increased release of ATP from the urothelium of painful bladders as compared to normal situation. Adenosine triphosphate thus, may have an important role in the mechanisms governing abnormal bladder sensation. This study has suggested an increased purinergic activity in terms of an increased ATP release in painful bladders as compared to normal bladders. This would explain most symptoms resulting from this condition and may provide a target for the development of new therapies for different conditions that are included in this category.

CONCLUSIONS

There is a significantly increased level of ATP release from urothelium of painful bladders in comparison to normal bladders suggesting an important role for ATP in this condition. This study provides direct evidence for the increased purinergic activity in painful disorders of the bladder.

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Table 1: ATP release following mechanical stretch and electric field stimulation

	ATP release with Mechanical stretch (pM/gm tissue)			ATP release with Electrical stimulation (pM/gm tissue)	
	Baseline	130%	150%	Baseline	10 Hz
Painful bladder Urothelium	1321.1±475.7†	2944.6±565.9*	3791.4±667.9*	496.4±21.1†	1348.6±278.2*
Control	45.7±4.9	67.5±13.2	77.6±16.2	45.8±5.1	61.7±10.1

* $p<0.005$, compared with baseline value †$p<0.01$, compared with control urothelium

Table 2: Effect of TTX (5μM) on ATP release following electrical and mechanical stimulation of painful bladder and normal urothelium.

	ATP release with Mechanical stretch (150% elongation) (% Increase over baseline)		ATP release with Electrical stimulation (10Hz) (% Increase over baseline)	
	TTX absent	TTX present	TTX absent	TTX present
Painful bladder urothelium	182.2±24.8*	165.2±40.1†	730.4±45.8*	185.9±38.1‡
Control	75.4±21.7	60.9±20.1	37.9±4.4	8.7±2.2

*$p<0.005$, compared with control urothelium †$p=0.2$[NS] compared with TTX absent

‡$p<0.001$,compared with TTX absent

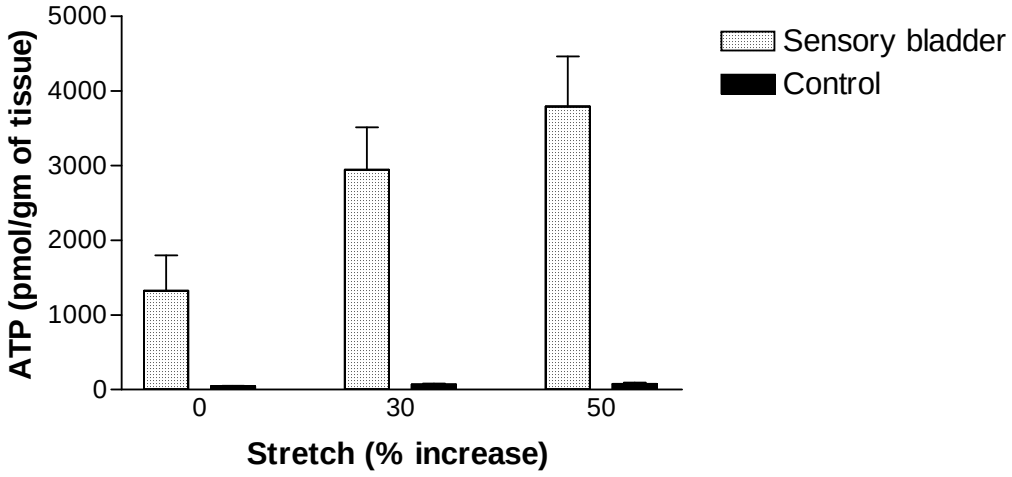

Figure 1. ATP release following mechanical stretch of painful bladder urothelium vs. normal urothelium ($p<0.005$).

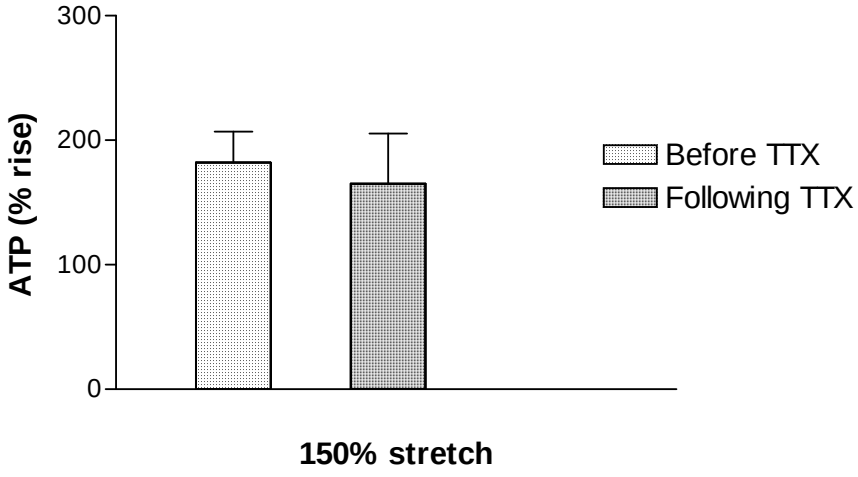

Figure 2. Bar chart showing relative reduction in ATP release from the urothelium of painful bladder, following TTX on mechanical stretch (p=NS).